REMARKS

The Office Action, dated July 7, 1999, has been carefully considered. The claims have been amended to more clearly set forth the Applicants' contribution to the art and do not introduce new matter into the disclosure of the invention. The basis for the amendments to the claims can be found on pages 10-18 and further on pages 23-33 of the Specification. It is believed that no additional fee is required as the number of independent and dependent claims is the same as originally filed.

For the sake of clarity, Applicants wish to reinforce the meaning of various terms used in this response, as used in USSN 09/167,088 (the instant application), and in US Patent Nos. 5,328,899 (*Tamarkin et al.*) and 5,612,034 (*Pouletty et al.*).

The Examiner has requested that a new declaration, including the application serial number and filing date, be submitted. Applicant respectfully submits that such a new declaration is not required. The declaration originally filed (copy attached) was attached to the application and, as such, did not need the serial number and filing date to identify the application it corresponds to. There is no requirement in 35 U.S.C. 115 or 37 C.F.R. 1.63 that a declaration include such information unless it is filed separately from its corresponding application.

Rejections under 35 U.S.C. 112, Second Paragraph

The Examiner has rejected claims 1-42, under 35 U.S.C. 112, second paragraph, contending that they are indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The claims have been amended herein to address the issues raised by the Examiner.

Specifically, the Examiner contends that claim 1, step a, has improper antecedent basis in reciting "injecting a human." Claim 1 has been amended to "injecting the human" for proper antecedent basis.

The Examiner contends that claim 1, step a, is indefinite in its use of the word "appropriate" in the phrase "an appropriate amount of targeting moiety." The word "appropriate" has been deleted to make the claim definite.

The Examiner contends that claim 1, step b, is indefinite in its use of the word "sufficient." This objection is respectfully traversed. The word at issue is used in the phrase "time sufficient to bind the target analyte of interest." Therefore, the context in which the word is used clearly defines what it means. One skilled in the art should have no problem determining whether a "sufficient" time has passed based on whether the target analyte has been bound by the targeting moiety.

The Examiner contends that claim 1, step b, is indeterminate in reciting " without dissociation of the target analyte from the targeting moiety." Since the previous step already states that the targeting moiety and the target analyte have conjugated, this phrase has been deleted.

The Examiner contends that claim 1, step e, lacks antecedent basis in reciting "the assay mixture". Step e has been amended to recite "the assay mixture of step d" for proper antecedent basis. The Examiner also contends that there is insufficient antecedent basis for the limitation "immobilized capture moiety" in step e. The word "immobilized" has been deleted to correct antecedent basis. Furthermore, the Examiner contends that claim 1, steps e and f, are indefinite in reciting "the immobilized capture moiety to bind specifically to either the target analyte or the labeled targeting moiety" since step b indicates the complex formation. Therefore, the phrase "either the target analyte or the labeled targeting moiety" is replaced with "the target moiety: target analyte conjugate".

The Examiner has rejected claim 1, under 35 U.S.C. 112, second paragraph, contending that claim 1 is incomplete for omitting an essential step of detection of the target analyte using detection labels. Therefore, step g has been amended to read "detecting bound targeting moiety: target analyte conjugate on the capture moiety using one or more detection labels".

The Examiner contends that claim 8 recites overlapping Markush language and is indeterminate in scope by reciting "paratopic molecules, recombinant molecules of binding sites". Claim 8 has been amended to delete the words "paratopic molecules" to clarify this claim.

The Examiner contends that claims 20, 22, 23 and 24 are indefinite and inconsistent as to the correlative relationship between "another molecule" in claim 20, "the molecule capable of binding... is an antibody" in claim 22, "the polyclonal antibody" in claim 23, and "the capture moiety is an antibody" in claim 24. Claims 20, 22, and 23 have now been amended to provide consistency throughout the claims by amending the claims to describe the "targeting moiety" and "another molecule" as a "first" and "second" targeting moiety.

The Examiner contends that there is insufficient antecedent basis in claim 25 for the limitation "detecting the bound conjugate on the *solid support*". Claim 25 is now been amended to delete the phrase "on the solid support."

The Examiner contends that claims 34 and 37 are indefinite in reciting the term "useful" for being a relative term that has no comparative basis for defining its metes and bounds. These claims have now been amended by providing the term "for" instead of "useful in".

The Examiner contends that claim 34 is indeterminate in scope by reciting "targeting moiety specific for the target analyte" and "contains the standard for the analyte" since the claim does not specifically identify the metes and bounds of the "target analyte". Step (c) of claim 34 has now been amended to recite "contains a standard for the target analyte."

Based on the foregoing amendments and remarks, it is submitted that the 35 U.S.C. 112 rejections have been overcome and it is respectfully requested that they be withdrawn.

Rejection under 35 U.S.C. 103

Examiner has rejected claims 1-42, under 35 U.S.C. 103, as being unpatentable over Tamarkin *et al.* (US 5,328,899), in view of Finkelman *et al.* (Journal of Immunology 151:1235-1244 (1993)) and in further view of Pouletty *et al.* (US 5,612,034). It is Applicants' position that these references fail to present a prima facie case of obviousness.

The present invention, as defined by amended claim 1, relates to a method for measuring the endogenous levels of target analyze, such as a cytokine. The present invention provides the capability of measuring basal, as well as stimulated, cytokine production.

In proceedings before the Patent and Trademark Office, the examiner bears the

burden of establishing a prima facie case of obviousness based upon the prior art. *In re Piasecki*, 745 F.2d 1468, 1471-72, 223 USPQ 785, 787-88 (Fed. Cir. 1984). The examiner can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). Indeed, the teachings of references can be combined only if there is some suggestion or incentive to do so. *ACS Hospital Sys., Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984).

It is not clear how any of the cited references can be seen to provide the necessary specific motivation, much less a reasonable expectation of success, when none of these references address the problems which are solved according to the present invention.

The assay disclosed by Tamarkin reference is different than the present invention. The Tamarkin assay is a competitive binding assay where polyclonal antibodies are adhered to a plate. The assay measures the ability of an analyte present in a serum sample to block the binding of biotin-labeled analyte to the plate. In such an assay, if the bodily fluid contains a large amount of analyte, most or all antibody adhered to the plate is bound by the analyte so that the biotin-labeled analyte is unable to bind to free antibody. As the amount of analyte present in the biological fluid increases, the biotin-labeled cytokine binding decreases.

Conventional immunoassays, in particular enzyme immunoassays, are well known in the art. The Tamarkin reference merely teaches a competitive form of the enzyme linked immunosorbent assay (ELISA) to measure hormones and growth factors in biological fluids. The manner by which an unknown ligand, such as a cytokine, is detected is similar to that of a competitive radioimmunoassay. Briefly, a specific amount of a labeled analyte, for example, labeled biotinylated cytokine, is in competition with unlabeled cytokine (either in the unknown sample or in a standard) for a limited number of antibody binding sites.

In the first step of the Tamarkin assay, an antibody, preferably a polyclonal antibody that recognizes many epitopes on the cytokine molecule, is adsorbed to a solid phase support or carrier. This antibody, known as a "capture antibody," is then used to bind the labeled analyte and the unlabeled analyte in the sample or the standard. After appropriate washing steps, an enzyme-conjugated binding partner for the label is incubated with the antibody-analyte complex, allowing the enzyme to be bound to the complex. After removal of any unbound enzyme-conjugated binding partner, a chromogenic enzyme substrate is added. The bound enzyme converts the substrate to a colored product that can be detected by colorimetric means. The amount of color that develops per unit time is inversely proportional to the amount of analyte present in the sample. As the concentration of analyte increases, the amount of color generated decreases.

The assay used in the Tamarkin reference measures the total of bound and unbound analyte present in a biological fluid. If the analyte is one with a short biological half-life and is not bound by endogenous serum proteins, the Tamarkin assay binding protein would not contact the analyte at all since it would be used up too quickly in the biological system. If endogenous serum proteins bind the analyte, then the methods of the Tamarkin reference would measure some product of the quantity of the analyte and quantity of analyte binding protein produced over an unknown period of time. Differences in the quantity of analyte measured in two samples might reflect differences in quantity of analyte binding protein in the samples rather than quantity of analyte produced.

In order to measure the amount of analyte at any given instance, one must inject an amount of binding molecule that is considerably in excess of the quantity of analyte produced in order to not saturate the binding of the analyte. If the binding protein is saturated, one measures only the amount of the analyte binding proteins present, not the amount of the analyte itself.

Additionally, there is no evidence in the Tamarkin reference that the biological half-life of the analyte binding protein used is longer than the analyte itself. The half-life of the analyte binding proteins must be considerably longer than the analyte of interest in order to use the present methods. It is not clear that the analyte binding proteins naturally present block utilization of the analyte. In the present invention, the binding proteins must be a neutralizing binding molecule, such as an antibody or soluble receptor, to block

utilization. Claim 1, step a, has now been amended to define the targeting moiety as a "neutralizing targeting moiety ... , at a concentration in excess of measurable quantities of secreted analyte." (antecedent basis at page 18, lines 9-12, 22, and 23; and page 21, lines 7-9) A "neutralizing" targeting moiety has been defined in the specification as a molecule that binds to the analyte and prevents its catabolism, excretion, or binding to its respective receptor. The neutralizing targeting moiety causes the analyte, which normally has a very short in vivo half-life, to accumulate in vivo as a targeting moiety:target analyte conjugate. In general, the neutralizing targeting moiety protects and preserves molecules that are produced in vivo, and that would ordinarily have a short in vivo half-life, by having a longer in vivo half-life. These differences clearly distinguish the present invention over Tamarkin.

Finally, Tamarkin discloses that some cytokines are present in complexed forms where some antigenic sites are covered and cannot be bound by antibodies. To get around this feature, many polyclonal antibodies are used so that at least one binding site may be available for binding to one of the antibodies. The present invention has great specificity because it uses two binding molecules (the targeting moiety and the capture moiety) that are preferably monoclonal antibodies. It is unlikely that both monoclonal antibodies would attach to a common unknown molecule to yield an incorrect measurement. Because Tamarkin uses a single polyclonal antibody, there is a good chance that the antibody will bind more than one analyte. This greatly decreases the specificity of such an assay.

To summarize, Tamarkin differs from the claimed process in that it is a competitive binding assay using polyclonal antibodies adhered to a plate to measure the ability of an analyte present in a serum sample to block the binding of biotin-labeled analyte to the plate. The present invention, however, is technique for evaluating in vivo cytokine production through the in vivo capture of secreted cytokines by labeled analyte-binding reagents, followed by in vitro measurement of serum levels of the captured analyte. No other technique has been described that allows quantitation of the amount of a biological material that has been secreted over a fixed, definable period of time in vivo.

The Tamarkin reference teaches using a single, polyclonal antibody, not two specific binding molecules (preferably monoclonal antibodies); it is not utilized in vivo to obtain the specific amount of analyte excreted over a fixed period of time; it does not teach using an excess of binding molecule; and it does not teach using a neutralizing binding molecule that binds the analyte and prevents its catabolism, excretion, or binding to its respective receptor.

Even assuming, *arguendo*, it were appropriate to combine Finkelman and Pouletty with Tamarkin, these references do not bridge the gap between Tamarkin and the present invention.

The Finkelman *et al.* reference discloses the idea that if one complexes an analyte, such as a cytokine, with an analyte binding protein and then injects the complex, the analyte survives longer in vivo than free or unbound analyte. By doing this, one does not

interfere with analyte utilization because the analyte is slowly released. The analyte binding in the Finkelman reference is not totally stable so the complex slowly releases the bound analyte in vivo. One would then expect that if you employ an anti-analyte antibody, such complex could not be used to measure analyte production.

In the present invention, however, the novel approach is to inject large quantities of analyte binding molecule so that the analyte binding molecule is in excess in order to favor analyte binding rather than dissociation and allow for more reliable capture and measure of such analyte. The result is that the analyte-binding molecule acts to capture the analyte and blocks utilization versus the association and concurrent slow release of the Finkelman reference.

The Finkelman reference uses an insufficient quantity of analyte binding molecule to sufficiently bind all of the analyte (a ratio of analyte to analyte binding molecule of 2:1). It does not utilize an excess of binding molecule, as is required by the present claims. Under such assay conditions, the analyte-binding molecule, such as an antibody, is saturated with bound analyte. In the present invention, the analyte be saturated in its binding to the analyte binding molecule. In the present invention, the user partially blocks utilization of the analyte but does not block utilization so completely as to prevent the natural immune response from occurring. Neither the Finkelman reference, nor any other reference, can be seen to provide the necessary specific motivation, much less a reasonable expectation of success, that one could inject enough analyte binding molecule that would

allow measurement of the analyte yet still be less than the amount that would block the natural immune response in the host.

Pouletty *et al.* teaches only that one can increase the in vivo biological half-life of a compound that normally has a short in vivo half-life by injecting it into an animal so that it binds covalently to a molecule that naturally has a long in vivo half-life. This merely points out a method of increasing the half-life of an injected compound not an endogenous compound. In the present invention, injecting a binding molecule that has a long biological half-life increases a biological half-life of a naturally occurring compound that has a short half-life. Also, in the preferred embodiment of the present invention in claim 8, the paratopic molecules used bind to the naturally occurring compound through noncovalent, rather than covalent, bonds.

Finkelman and Pouletty do not lend the necessary elements of the present invention that are missing in Tamarkin. Therefore, even if combined, the invention of claim 1 is not taught. Specifically, Finkelman only teaches that one may prolong the half-life of a fast metabolizing molecule by binding it to a slow metabolizing molecule. But, it teaches to use an unstable binding to yield a slow release of the analyte. One could not measure the quantity of analyte under these conditions. Additionally, it does not teach the use of an excess of binding molecule. Pouletty only teaches that the in vivo half-life of a exogenous molecule may be increased by binding it to a molecule with a long biological half-life. It does not describe using endogenous compounds. And Pouletty, as with Finkelman and

Tamarkin, does not teach using two binding molecules, an excess of binding molecule, or use of a neutralizing binding molecule.

Accordingly, lacking a technological rationale, the reasonable expectation of success, and motivation, it is not seen how any combination of the cited references could establish a prima facie case of obviousness. Furthermore, even if a prima facie case of obviousness exists, the long felt need and unexpected advantages of Applicants' invention clearly overcome this.

As the Tamarkin reference indicates (which the Examiner has relied upon), there is wide variation in the reported results with regard to cytokine concentration in the blood and to fluctuations of cytokine concentration in the blood and many reports indicate that cytokines (i.e., IL-2) are not detectable in normal subjects using immunoassays. Furthermore, there is currently no reliable method of measuring endogenous analyte production by sampling biological fluid although there has been such an obvious, overwhelming need for endogenous cytokine measurement and all of the components used in Applicants' invention have been commercially available for years. All in all, the secondary indications all point to the nonobviousness of the present invention.

It is, therefore, respectfully requested that the rejection under 35 U.S.C. 103 be withdrawn. In view of the above, it is respectfully submitted that the claims as amended and presently before the Examiner are in condition for allowance. Accordingly,

reconsideration and withdrawal of the rejections and objections are requested and allowance of claims 1 through 42 is solicited.

Respectfully submitted,

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The following papers were received in the United States Patent & Trademark Office:

Applicant: Finkelman et al.
Title: Methods for measuring in vivo cytokine production
Ser. No. 09/167,088 Filed: October 6, 1998

1. Amendment & Remarks under 37 CFR 1.115